J Pest Sci (2010) 83:27–32
DOI 10.1007/s10340-009-0265-x

ORIGINAL PAPER

Effect of botanical insecticide Nimbecidine® on food consumption and egg hatchability of the terrestrial snail *Monacha obstructa*

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Received: 7 December 2008 / Revised: 29 June 2009 / Accepted: 30 June 2009 / Published online: 28 August 2009

Abstract In the laboratory, a commercial neem-based insecticide—Nimbecidine®—was evaluated as a potential pest management tool for the terrestrial snail, *Monacha obstructa* (Pfeiffer, 1842) (Hygromiidae). Effects of different concentrations of the botanical insecticide on food consumption and egg hatchability of the terrestrial snail were studied. Generally, food consumption of immature and adult snails decreased as the concentrations of Nimbecidine® increased. At the highest concentration (10 ml/l), the snails avoided contacting with food completely. The food intake of immature individuals was significantly ($p < 0.05$) more affected by the Nimbecidine® treatment (at 1.25 ml/l) than that of adults. LC_{50} of Nimbecidine® for the treated eggs was 2.18 ml/l, and eggs failed to hatch at concentration of 10 ml/l. Nimbecidine® showed sufficient biological activity against the food consumption and eggs viability of *M. obstructa*, thus the preparation has a potential to protect field crops from this pest snails.

Keywords Neem oil · Antifeedant effect · Ovicidal effect · Hygromiidae · *Monacha obstructa*

Introduction

Terrestrial snails are major pests of arable crops due to their ability to hollow grains and destroy newly emerged shoots and leaves. They cause considerable economic damage to a wide variety of plants including vegetables, tree fruits and ornamental plants (Nakhla et al. 2002; Ismail et al. 2003). Current control measures against the snails rely mainly on the use of bait pellets containing a molluscicide, primarily methiocarb and metaldhyde (Garthwaite and Thomas 1996). Although such pellets are effective, the active ingredients are also implicated in the poisoning of non-target organisms (South 1992). This is particularly problematic where the non-target organisms themselves are involved in the pest control. For example, non-target insects, such as carabid beetles, which are known to be important natural control agents of slugs and snails, can die after ingestion of methiocarb-containing pellets (e.g. Purvis and Bannon 1992; Langan et al. 2004; Toor 2006).

Pest management is moving towards practices that reduce reliance on conventional pesticides and using pesticides from natural sources, such as plants or microorganisms, is desirable. Recently, it has been shown that many organisms are sensitive to neem extracts. These include insects from several orders, and also mites, nematodes, snails, fungi and viruses (Bhatnagar et al. 1990; Locke 1990; National Research Council 1992). Neem (*Azadirachta indica* A. Juss) is a tree native to India (Roxburgh 1874), which has been successfully established in other tropical and subtropical areas in Asia, West Africa, Australia and South America (Srivastava et al. 1997). Until now, four biologically active compounds in neem extracts have been shown to be highly effective in their activity as pesticides: azadirachtin, salannin, meliantriol and nimbin (Jacobson 1990). Azadirachtin is a mixture of seven isomeric compounds labelled as azadirachtin-A to azadirachtin-G with azadirachtin-A being present in the highest quantity and azadirachtin-E regarded as the most effective insect growth regulator (Verkerk and Wright 1993). The biologically

Communicated by M. Traugott.

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active compounds can be extracted by several methods. Leaching with water is the oldest method and is still used to selectively extract azadirachtin. On the other hand, most companies are using more non-polar solvents to obtain a more varied mixture of chemicals (Lee et al. 1988; Schmutterer 1990; National Research Council 1992). Neem extracts are often described to have minimal toxicity to beneficial organisms such as parasitoids, predators and pollinators (e.g. Lowery and Isman 1995; Naumann and Isman 1996; Raguraman and Singh 1999) and can degrade rapidly in the environment (Isman 1999). The extracts are also relatively safe to carabid beetles, the main predators of snails (e.g. Forster 1991; Mohapatra et al. 1991; Srinivas and Madhumathi 2005). Generally, the neem extracts can have various effects on living organisms. Antifeedant and growth regulating effects of neem extracts are the most valued in the pest management as these are the most intense on the widest range of insects. Other secondary effects that have been studied include repellency, antioviposition, fecundity reduction, loss of flying ability, sexual communication disrupting and guttural motility reduction (Schmutterer 1990; National Research Council 1992; Mordue 2004).

In Ismailia Governorate, terrestrial snails are active in the fields from the end of September to the beginning of June. *Monacha obstructa* (Pfeiffer, 1842) (Hygromiidae) is the most common snail species on cultivated crops and it is recorded in high population density on Egyptian clover, cabbage, green beans, maize and cucumber (Shoieb 2008). Although molluscicidal effects of several plant-derived extracts on terrestrial and aquatic snails have already been studied (e.g. Gabr et al. 2006; Sangita et al. 2006; Pallabi et al. 2007), little is known about ovicidal and antifeedant effects of neem oil on *M. obstructa*. Therefore, the aim of this study was to determine effects of a commercial neem oil-based botanical insecticide Nimbecidine® (containing 0.03% azadirachtin and other limonoids like meliantriol, salannin, nimbin and other terpinoids in the ratio as it occurs in neem in nature) on food consumption and egg hatchability of this pest snail species.

Materials and methods

Collecting of snails

Specimens of the terrestrial snail, *M. obstructa* were collected from infested Egyptian clover in Ismailia Governorate, Egypt. Samples were collected by hand picking and collected snails were kept in plastic containers at 25 ± 2°C, 75–85% RH and photoperiod of 12/12 h (L/D). The rearing containers ($160 \times 110 \times 60$ mm^3) were lined with sterile loam soil on the bottom and covered with mosquito netting to provide ventilation. The snails were allowed to acclimatize to the laboratory conditions for 4 days and they were fed fresh cabbage leaves ad libitum daily. Egg laying was observed each day and all egg clutches deposited in the rearing containers were carefully collected for the ovicidal effect experiment. All individuals intended for the food consumption experiment had been starved for 24 h prior to the bioassay.

Food consumption experiment

A neem oil-based botanical insecticide Nimbecidine® (containing azadirachtin (0.03%) as well as other limonoids like meliantriol, salannin and nimbin in the ratio as it occurs in neem in nature) was obtained from T. Stanes & Company Ltd (Coimbatore, India). Pesticide solutions were prepared in distilled water according to producer's instructions. Doses were administered to obtain four final concentrations of 10, 5, 2.5 and 1.25 ml/l. In a control variant, distilled water without Nimbecidine® was used. Food consumption of adult and immature animals was tested separately. The snail stages were discriminated according to main morphological characters (diameter of shell and number of shell coils). The shell diameter of mature individuals used in the bioassay ranged between 11 and 13 mm and the shell diameter of immature ones ranged between 8 and 9 mm. Fifty individuals of either age category were used for each Nimbecidine® concentration. The experimental animals, divided into five repetitions (each of 10 animals), were exposed to food contaminated with the pesticide solutions. Additional batch of 10 snails was used in the negative control variant. Leaves of lettuce (*Lactuca sativa* L.) were used as food in the experiment. Leaf discs of 5 g were used for each repetition. The leaf discs were dipped into the test solutions for 5 s with gentle agitation, allowed to surface-dry on a paper towel and placed into the rearing plastic boxes with the experimental animals. The experiment was carried out in an environmental chamber at 25 ± 2°C, 75–85% RH and 12/12 h (L/D) for 7 days. To determine daily food consumption, after each 24 h, the remains of food were removed from rearing boxes and fresh food (5 g), treated with the pesticide solutions as mentioned above, was added to the boxes. Each day, fresh pesticide solutions were mixed and used for a food treatment. Weight of the food remains was measured to determine daily food consumption of tested animals.

Ovicidal effect experiment

Clusters of eggs laid under laboratory conditions were collected by a fine hair brush. The eggs were divided into batches of 10 eggs for ovicidal test and each batch of eggs (up to 24 h old) was placed into a Petri dish (40 mm diameter)

Table 1 Effect of Nimbecidine® on food consumption of *M. obstructa* adults under laboratory conditions

Nimbecidine® concentration	Mean weight of food consumed on days 1–7 of bioassay (g)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
10 ml/l	0.00 a*	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
5 ml/l	0.72 b	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
2.5 ml/l	0.86 b	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
1.25 ml/l	0.46 ab	1.38 b	1.98 b	1.45 ab	1.94 b	1.78 b	1.78 b
Control	3.98 c	4.98 c	2.99 c	3.99 c	2.98 c	3.33 c	4.95 c

* Values followed by the same letter in a column are not significantly different (FLSD test, $p < 0.05$)

containing 5 g of sterile moist soil. Altogether, five different concentrations of Nimbecidine®, namely 10, 5, 2.5, 1.25 and 0.625 ml/l with a negative control variant (distilled water without Nimbecidine®) were prepared and 2 ml of each pesticide solution were topically applied directly onto egg batches. Five repetitions (each of 10 eggs) and the negative control were used for each azadirachtin concentration. The experiment was maintained at the same temperature, humidity and light regime as the food consumption bioassay. Number of hatched eggs was recorded daily for 2 weeks and percentage of egg hatchability was calculated.

Statistical analysis

One-way ANOVA (SAS Institute Inc. 1999) was used to determine significant differences amongst pesticide treatments and mean weight of food consumed on days 1–7 of bioassay. Data on food consumption of immature and adult snails were subjected separately to ANOVA. One-way ANOVA was also used to determine differences in egg hatchability amongst Nimbecidine® concentrations used. If there were significant differences ($p < 0.05$), Fisher's least significant difference mean separation test (FLSD) was used. For the survival tests of snail eggs, lethal concentrations LC_{20}, LC_{50} and LC_{90} were determined using a standard probit analysis.

Results

Data presented in Tables 1 and 2 show the effect of Nimbecidine® on food consumption of *M. obstructa*. Nimbecidine® caused reduction in the food consumption of immature and adult snails compared with untreated control variants. It was observed that the repellent activity of Nimbecidine® was dose dependent. At the highest concentration (10 ml/l), the adult and immature snails avoided contacting with food completely. The lower concentrations affected the food consumption of tested animals. The food consumption was significantly higher ($p < 0.05$) in the control variants than in all of the treatments with Nimbecidine® for both age categories of snails. Correlation between Nimbecidine® concentration and the food consumption on the first day of bioassay was significant ($r = -0.74$, Pearson correlation analysis, $p < 0.05$) for the adult stage. At concentrations of 2.5 and 5 ml/l, the adult and immature snails did not consume any lettuce on the second day of experiment. At the lowest concentration tested (1.25 ml/l), the adult and immature snails continued in food consumption till the end of the experiment; however, the food intake was significantly reduced in comparison with the control, on average, by 33.78–88.44% ($p < 0.05$) and 94.45–100% ($p < 0.05$) for adult and immature snails, respectively. The food intake of immature individuals was significantly ($F_{4.74} = 47.34$, $p < 0.05$) more affected by the Nimbecidine® treatment (at 1.25 ml/l) than that of adults. On average, the food consumption of immature individuals was affected more than that of adults by 37.54%. Food consumption in the control variant was not significantly different between immature and adult snails ($F_{4.74} = 0.03$, $p > 0.05$).

The hatchability of *M. obstructa* eggs treated with Nimbecidine® is shown in Table 3. The results show that the highest concentration of Nimbecidine® (10 ml/l) caused 100% mortality of eggs. At lower concentrations, the average hatchability was between 20–50% according to the concentration level. Mean values of Nimbecidine® lethal concentrations LC_{20}, LC_{50} and LC_{90} for the eggs were 0.80 (0.50–1.28; 95% confidence interval) ml/l, 2.18 (1.71–2.78) ml/l and 10 (6.88–14.52) ml/l, respectively.

Discussion

Molluscicidal activity of neem-based products has already been documented. This plant-derived pesticide was tested separately or in combinations with other herbal extracts, commercial pesticides or adjuvants (e.g. Singh et al. 1996, 1998; Rao and Singh 2000; Singh and Singh 2000, 2001; Iglesias et al. 2002a, b; Pal et al. 2002; Hollingsworth and Armstrong 2003; Pallabi et al. 2007). Antifeedant activity of neem preparations has also been tested against terrestrial and aquatic snails; however, the results are inconsistent and

Table 2 Effect of Nimbecidine® on food consumption of immature *M. obstructa* individuals under laboratory conditions

Nimbecidine® concentration	Mean weight of food consumed on days 1–7 of bioassay (g)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
10 ml/l	0.00 a*	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
5 ml/l	0.18 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
2.50 ml/l	0.08 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
1.25 ml/l	0.19 a	0.26 a	0.24 a	0.00 a	0.00 a	0.00 a	0.22 a
Control	4.67 b	4.95 b	2.97 b	3.98 b	3.68 b	3.48 b	3.97 b

* Values followed by the same letter in a column are not significantly different (FLSD test, $p < 0.05$)

Table 3 Effect of Nimbecidine® (0.03% azadirachtin) on egg hatchability of *M. obstructa* under laboratory conditions

Nimbecidine® concentration	Mean egg hatchability (mean ± SE) (%)
10 ml/l	0 a*
5 ml/l	10 ± 4.47 a
2.5 ml/l	32 ± 5.83 b
1.25 ml/l	44 ± 2.45 c
0.625 ml/l	64 ± 5.10 d
Control	100 e

* Values followed by the same letter in a column are not significantly different (FLSD test, $p < 0.05$)

differ according to snail species. In most experiments, these preparations negatively affected the food intake of snails, but phagostimulant effect was detected as well. For instance, a terrestrial snail, *Zonitoides arboreus* (Say, 1817), fed lettuce treated with neem extracts consumed significantly more lettuce than control. This effect occurred both for neem oil and a chemical pesticide having azadirachtin as the active ingredient (Hollingsworth and Armstrong 2003). It is evident from our results that Nimbecidine® has antifeedant effect against *M. obstructa* and the effect was dose dependent. Of the biologically active ingredients of neem oil-based pesticides, azadirachtin, meliantriol and salannin are the main compounds that reduce feeding of many species of pest insects (Mordue 2004). Since Nimbecidine® contains a complex of limonoids (azadirachtin (0.03%), meliantriol, salannin and nimbin), our results do not demonstrate which of these compounds are most responsible for the effect observed. Synergistic effect of the compounds can be expected. During our research, molluscicidal activity of Nimbecidine® to *M. obstructa* was not observed even at the highest concentration; however, in other experiments, a different neem-based preparation (Neemix 4.5®) did show molluscicidal activity (Gabr et al. 2006).

Singh and Singh (2000), while studying the effect of *A. indica* on the reproduction of the aquatic snail *Lymnaea acuminata* Lamarck 1822, reported that the active molluscicidal constituent (azadirachtin) caused a significant reduction in egg viability. In our experiments, the hatchability of *M. obstructa* eggs was greatly affected with Nimbecidine®. The highest concentration of Nimbecidine® (10 ml/l) caused 100% mortality of eggs, and at lower concentrations, the average hatchability decreased to 20–50%. In the control, no egg mortality was observed and the LC_{50} for the eggs was 2.18 ml/l of Nimbecidine®. Ovicidal activity of neem oil was also documented on eggs of the snail, *Biomphalaria alexandrina* (Ehrenberg, 1831) (Mostafa and Abdel-Megeed 1996). The authors found that ovicidal effects of neem oil against *B. alexandrina* eggs decreased with stage of development of the eggs. All eggs used in our experiments were of the same developmental stage, thus our results cannot confirm this phenomenon.

We found that Nimbecidine® did not show ability to kill the snails; however, it showed a strong antifeedant effect against the snail and caused 100% inhibition of its feeding activity at a dose of 10 ml/l. It can be concluded from this study that Nimbecidine®, when used at product recommended rate (5 ml/l), showed significant biological activity against food consumption and egg viability of *M. obstructa*. Although biological activity has not been studied under field conditions in our experiments, the results suggest that Nimbecidine® can have a potential to protect field crops from *M. obstructa*. Nimbecidine® activity has already been successfully documented under field conditions in various crop–pest systems (e.g. Singh and Singh 2007; Jandial and Kamlesh 2008; Patil 2008). Viability of snail eggs, which are laid in the superficial layer of soil, can also be effectively reduced by application of this preparation. Therefore, the strategy of snail control based on killing eggs also has the potential in organic agriculture. Although further field studies are necessary, it is our belief that the use of neem-based pesticides against this harmful snail would achieve effectiveness comparable to synthetic molluscicides.

Acknowledgment The authors thank T. Stanes & Company Ltd, Coimbatore, India for providing us with a free sample of Nimbecidine® for this research work.

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